CON7.

28. An isolated non-toxic, non-toxigenic, non-pathogenic *Fusarium venenatum* host cell, having the identifying characteristics of *Fusarium venenatum* ATCC 20334, comprising a nucleic acid sequence encoding a heterologous protein.

## **REMARKS**

Claims 23-25 have been canceled. New claims 26-28 have been added and are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

# 1. The Rejection of Claims 23-25 under 35 U.S.C. § 112, First Paragraph

Claims 23-25 stand rejected under 35 U.S.C. § 112, first paragraph, "because the specification, while being enabling for the use of cells deposited at ATCC under deposit number 20334, does not reasonably provide enablement for the use of recombinant host cell having the identifying characteristics of a non-toxic, non-toxigenic, non-pathogenic *Fusarium venenatum* host cell." The Office Action specifically states:

The specification is silent with respect to what identifying characteristics enable correct identification of *Fusarium venenatum*. Colony morphology of *Fusarium graminearum* on PDA are specifically provided which ultimately are not sufficient for *Fusarium venenatum*. As we are taught in Yoder *et al.* morphological characteristics are best discernible between strains by growth on minimal media. Cultural characteristics recited in the specification most closely resemble *Fusarium graminearum* and not *venenatum*. Required techniques for the proper execution of identifying *Fusarium venenatum* as well as critical primers and unique cultural details are not provided in the specification. Therefore, no disclosure for a critical aspect of the invention, host cells having the identifying characteristics of a non-toxic, non-toxigenic, non-pathogenic *Fusarium venenatum* host cell is provided.

This rejection is respectfully traversed.

The Office Action asserts that the skilled artisan would have to conduct undue experimentation and excessive experimentation in order to practice the claimed invention because the specification is silent with respect to what identifying characteristics enable correct identification of *Fusarium venenatum*. Applicants disagree with this assertion for several reasons.

First, Applicants respectively point out that taxonomic identification is an evolving area of expertise. As methodologies improve, taxonomic identification improves. Taxonomic identification is

handled by experts known in the art. While at the time of filing of the instant application, there were no published reports of *Fusarium venenatum* strains, the state of the art was in place to identify *Fusarium* strains as belonging to the species *venenatum*. H.I. Nirnberg, a world-renowned taxonomist, had submitted a paper on September 8, 1993 for publication in the journal *Mycopathologia*. Please refer to Nirenberg in *Mycopathologia* 129: 131-141, 1995; see Appendix H of Dr. Yoder's Declaration of April 19, 2000. Applicants submit that the state of art in taxonomic classification of *Fusarium venenatum* strains at the time of filing of the application was in place prior to September 8, 1993, as is described in the Nirenberg reference, which was submitted for publication on September 8, 1993. In fact, *Fusarium* ATCC 20334 was submitted blind to the laboratory of Nirenberg for taxonomic classification, after the filing date, which correctly identified the strain as *F. venenatum*. Since the capability to identify a strain as a *Fusarium venenatum* was therefore in existence by a major laboratory involved in fungal taxonomic classification at the time of filing, one of ordinary skill in the art could properly identify a *Fusarium venenatum* strain to determine whether the strain falls within the scope of Applicants' invention.

Second, the Office Action suggests that cultural and/or morphological characteristics are not reliable in identifying strains. Applicants respectfully disagree with the Office Action's assertion that the initial misidentification of the strain designated ATCC 20334 as F. graminearum implied that species and strain designations based solely on cultural and/or morphological characteristics are not reliable, consistent or accurate. In taxonomic classification, cultural and/or morphological characteristics are routinely used to determine the classification of a strain. Please refer to Nirenberg in Mycopathologia 129: 131-141, 1995, which clearly shows that such characteristics are reliable. As noted above, Fusarium strain ATCC 20334 was submitted blind to the laboratory of Nirenberg for taxonomic classification, which correctly identified the strain as F. venenatum. Nirenberg's taxonomic identification was performed as described in her paper Mycopathologia 129: 131-141, 1995. However, the Office Action asserts that "[a]s we are taught in Yoder et al. morphological characteristics are best discernible between strains by growth on minimal media. Cultural characteristics recited in the specification most closely resemble Fusarium graminearum and not venenatum." Applicants disagree with the Office Action's contention that "[c]ultural characteristics recited in the specification most closely resemble Fusarium graminearum and not venenatum" The characteristics described on page 4, line 26 to page 5, line 2, and page 5, line 24, to page 6, line 3, were describing the ATCC 20334 strain, which was really Fusarium venenatum, not Fusarium graminearum. Moreover, one of ordinary skill in the art would recognize the need to submit such a strain with such features and characteristics to an expert Fusarium taxonomist, such as Nirenberg, to confirm the strain is a Fusarium venenatum strain.

Third, the inherent characteristics and features of the ATCC 20334 strain did not change, just the species name. These inherent characteristics and features existed at the time of filing of the application and have not changed. Even though Nirenberg's paper did not publish until after the filing date, this should not limit the scope of protection of Applicants' invention because the knowledge existed in the art at the time of filing, as noted above. Supplemental methods in the art of taxonomy which were developed after the filing date, such as RAPD PCR, to confirm the identification of strains do not negate the reality that the identifying characteristics of *Fusarium* strain ATCC 20334 existed at the time of filing and have not changed. The Office Action indicates that Applicants' have not disclosed the identifying characteristics of a *Fusarium venenatum* strain. Applicants assert that a taxonomist skilled in the art could identify these characteristics as Nirenberg did prior to the date of September 8, 1993. Thus, ordinary skill in the art existed at the time of filing to accomplish the proper identification.

Fourth, the Office Action asserts that it would require undue experimentation to determine whether a *Fusarium venenatum* strain is non-toxic, non-toxigenic, and non-pathogenic. Applicants describe on page 5, lines 3-23, of the specification methods well known in the art for determining whether a strain is non-toxic, non-toxigenic, and non-pathogenic. One of ordinary skill in the art would have no difficulty in determining whether a *Fusarium venenatum* strain is non-toxic, non-toxigenic, and non-pathogenic.

Sixth, limiting the claims to *Fusarium* strain ATCC 20334 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another non-toxic, non-toxigenic, and non-pathogenic *Fusarium venenatum* strain having essentially the same properties as that of *Fusarium venenatum* strain ATCC 20334 and thereby circumvent the literal scope of Applicants' patent rights. For example, O'Donnell *et al.* in *Fungal Genetics and Biology* 23: 57-67, 1998 (see Appendix I of Dr. Yoder's Declaration of April 19, 2000), describe such a situation. O'Donnell *et al.* disclose in Table 3 on page 60 that *F. venenatum* NRRL 26139=BBA 64537 produces no toxins (see page 58 of reference).

Applicants assert, therefore, the specification, while being enabling for the use of cells deposited as ATCC 20334, also provides enablement for the use of recombinant host cells having the identifying characteristics of the non-toxic, non-toxigenic, non-pathogenic *Fusarium venenatum* host cell of ATCC 20334.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

# II. The Rejection of Claims 23-25 under 35 U.S.C. § 112, First Paragraph

Claims 23-25 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Office Action states:

The written description requirement for genus claims may be satisfied through sufficient description of a relevant representative number of species by actual reduction to practice or by disclosure of relevant identifying characteristics such as structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure or by a combination of such identifying characteristics sufficient to show applicants were in possession of the claimed genus. In the instant case, applicants present as relevant identifying characteristics, colony morphology of (ATCC 20334) Fusarium graminearum on PDA. However, this characteristic is not sufficient for Fusarium venenatum.

This rejection is respectfully traversed.

For the same reasons explained in section I (see above) and further reasons described below, Applicants assert that the subject matter is described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants preliminarily note that *Fusarium venenatum* strain ATCC 20334 has been deposited and is publicly available (see Section III below).

The Office Action contends that Applicants' disclosed examples for ATCC 20334 are not a representative number of species to show Applicants were in possession of the claimed genus. Applicants disagree with this contention because Applicants are not claiming the genus *Fusarium*, but rather a very specific species within the Section Discolor, *i.e.*, *Fusarium venenatum*. Moreover, Applicants further specify that the *Fusarium venenatum* must have the identifying characteristics of *Fusarium venenatum* strain ATCC 20334 and must be non-toxic, non-toxigenic, non-pathogenic. Applicants submit that the information disclosed in the specification combined with the knowledge in the art provides sufficient guidance to one of ordinary skill in the art to taxonically identify a non-toxic, non-toxigenic, and non-pathogenic *Fusarium venenatum* strain having the identifying properties as that of *Fusarium venenatum* strain ATCC 20334. Thus, there is sufficient written description in the specification to inform the skilled artisan that Applicants were in possession of the claimed subject matter at the time the application was filed.

Limiting the claims solely to *Fusarium* strain ATCC 20334 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another

non-toxic, non-toxigenic, and non-pathogenic *Fusarium venenatum* strain having essentially the same properties as that of *Fusarium venenatum* strain ATCC 20334 and thereby circumvent the literal scope of Applicants' patent rights. For example, as noted in Section I, O'Donnell *et al.* in *Fungal Genetics and Biology* 23: 57-67, 1998 (see Appendix I of Dr. Yoder's Declaration of April 19, 2000), describe such a situation.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35.U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

# III. Deposit of Fusarium ATCC 20334

The Office Action states that the *Fusanum venenatum* of the invention was apparently deposited by others and since its availability cannot be ensured, Applicants must deposit the specific *Fusarium venenatum* recited in the claims to satisfy a deposit requirement under 37 CFR 1.801-1.809. This request is respectfully traversed.

Under 37 C.F.R. 1.802(b), "[b]iological material need not be deposited, *inter alia*, if it is known and readily available to the public...." When the Patent Office adopted the rules on the deposit of biological materials, it issued comments on interpreting and applying the rules. See 54 FR 34880. The comments regarding the terms "known and readily available" in 37 C.F.R. 1.802(b) are set forth at page 23 of 54 FR 34880 (a copy thereof is enclosed herewith) as follows:

Even where access to biological material is required to satisfy these statutory requirements, a deposit may not be necessary if access sufficient to satisfy these requirements is otherwise available.

For example, applicant could show that the biological material is known and readily available to the public. The concepts of "known and readily available" are considered to reflect a level of public accessibility to a necessary component of an invention disclosure that is consistent with an ability to make and use the invention. To avoid the need for a deposit on this basis, the biological material must be both known and readily available - neither concept alone is sufficient....

By showing that a biological material is known and readily available or by making a deposit in accordance with these rules, applicant does not guarantee that such biological material will be available forever. Public access during the term of the patent may affect the enforceability of the patent. Although there is a public interest in the availability of the deposited material during and after the period of enforceability of the patent, the examiner need not be unduly concerned about continued access to the public. Unless there is a reasonable basis to believe that the biological material will cease to be available during the life of the patent, the examiner should accept current availability as satisfying the requirement. The incentives

provided by the patent system should not be constrained by the mere possibility that a disclosure that was once enabling would become non-enabling over a period of time through no fault of the patentee. *In re Metcalfe*, 410 F.2d 1378, 161 USPQ 789 (CCPA 1969) (emphasis added).

Applicants submit that the microorganism recited in the claims is "known and readily available" and, therefore, Applicants do not have to provide the assurances requested in the Office Action.

Applicants have enclosed a copy of page 178 of the ATCC catalogue for 1991 which lists Fusarium ATCC 20334. Also attached are pages from ATCC's website (<a href="www.atc.org">www.atc.org</a>) that disclose the strain is commercially available and may be purchased. Clearly, these publications establish that this strain was known prior to Applicants' filing date. Applicants note that they obtained the strain from this depository.

For the foregoing reason, Applicants submit that this rejection under 35 U.S.C. § 112 has been overcome and respectfully request reconsideration and withdrawal of the rejection.

## IV. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Date: October 24, 2002

Respectfully submitted,

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# Morphological differentiation of *Fusarium sambucinum* Fuckel sensu stricto, *F. torulosum* (Berk. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov.

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## Abstract

Within Fusarium sambucinum Fuckel sensu lato three species were differentiated: F. sambucinum Fuckel s. str., F. torulosum (Berk. et Curt.) Nirenberg comb. nov. and F. venenatum Nirenberg sp. nov. They are described and illustrated in detail.

Key words: Cultural characteristics, Fungi imperfecti. Fusarium sambucinum, Fusarium torulosum, Fusarium venenatum, Morphology, Taxonomy

# Introduction

Fusarium sambucinum Fuckel sensu lato is found quite often in the temperate zone and in the subtropics. It occurs in soil and on many plants. It is well known to plant pathologists for producing a dry rot on potato tubers in the field and in storage. For further details see Wollenweber [1], Domsch et al. [2], and Gerlach and Nirenberg [3]. This fungus also produces toxic secondary metabolites [4] such as diacetoxyscirpenol.

F. roseum Link is the type species of the genus Fusarium. Since the epithet roseum was used by Snyder and Hansen [5] in a completely different sense, F. sambucinum Fuckel being a synonym, replaced it. Therefore F. sambucinum is of special significance for Fusarium taxonomists. Wollenweber and Reinking [6] placed it in the section Discolor.

The present taxonomic views of Fusarium sambucinum Fuckel s. 1. are shown in Table 1: Wollenweber and Reinking [6] differentiated Fusarium sambucinum Fuckel, F. sambucinum Fuckel var. minus Wollenw. and five formae of which four were based solely on colour differences. Formae 1, 4 and 5 formed more slender sporodochial conidia than the basic species, whereas in formae 2 and 6 the width of the conidia did not differ from the latter. They combined forma 3 which had been described by Wollenwe-

ber with F. culmorum. They recognized F. trichothecioides Wollenw. as a seperate species.

Booth [7] accepted the varieties F. sambucinum var. sambucinum and F. sambucinum var. coeruleum as well as F. sulphureum Schlecht. and F. trichothecioides. F. sambucinum represented the red cultures on rich media like potato sucrose agar (PSA), F. sulphureum the yellowish and F. trichothecioides the rose coloured, pionnotal ones. He also thought that F. sarcochroum (Desm.) Sacc. was identical with F. sambucinum and F. bactridioides Wollenw. with F. trichothecioides.

Gerlach & Nirenberg [3] considered F. trichothecioides synonymous with F. sulphureum. Like Booth they accepted the two varieties F. sambucinum var. sambucinum and F. sambucinum var. coeruleum. Their concept of the variety coeruleum, like Booth's, was not the same as Wollenweber's and Reiking's. They described the strains of this variety (=f. 1) as having more slender conidia than those of the variety sambucinum, whereas according to Booth [7] and Gerlach and Nirenberg [3] the conidia are wider. They recognized F. bactridioides and F. sarcochroum as good species.

Nelson et al. [8] combined F. trichothecioides, F. bactridioides, F. sulphureum, F. sambucinum var. minus and F. sambucinum var. coeruleum with F. sambucinum without mentioning F. sarcochroum.

Table 1. Concept of Fusarium sambucinum Fuckel sensu lato by various authors

Wollenweber & Reinking 1935 [6]	Booth 1971 [7]	Gerlach & Nirenberg [982 [3]	Nelson et al. 1983 [8]	Nelson et al. 1983 [8] Nirenberg (present concept)
F. sambucinum Fuckel F. sambucinum f. I Wollenw. =F. sambucinum var. coeruleum	F. sambucinum ! F. sambucinum var. coeruleum <sup>b</sup>	F. sambucinum (all red strains)! ! F. sambucinum var. coeruleum	F. sambucinum F. sambucinum	F. sambucinum F. torulosum (Berk. et Curt.) comb. nov.
"Fusarium torulosum Berk. et Curt. ? F. culmorum (W.G. Sm.) Sacc. var. cerealis (Cook) Wollenw. pr. p. <sup>a</sup>			?F. culmorum	F. venenatum sp. nov.
F. sambucinum f. 2 Wollenw.	F. sambucinum var. coeruleum!c	F. sambucinum	F. sambucinum	F. sambucinum
F. sambucinum f. 4 Wollenw.	i	F. sambucinum!	F. sambucinum	F. torulosum
F. sambucinum f. 5 Wollenw.	i	F. sambucinum!	F. sambucinum	F. torulosum
F. sambucinum f. 6 Wollenw.	F. sulphureum	F. sulphureum	F. sambucinum	F. sambucinum
F. sambucinum var. minus Wollenw.	F. sambucinum	? F. sambucinum	F. sambucinum	? F. sambucinum
F. trichothecioides Wollenw.	F. trichothecioides	? F. sulphureum	F. sambucinum	F. sambucinum
F. bactridioides Wollenw.	F. trichothecioides	F. bactridioides?	F. sambucinum	F. bactridioides ?-
F. sarcochroum (Desm.) Sacc.	F. sambucinum	F. sarcochroum	i	F. sarcochroum?

<sup>2</sup>? In front of the taxon = author is not sure about synonymity.

b! In front of the taxon - taxon does not correspond with the original description.

c! After the taxon - author made an error by placing his concept of the taxon synonymous with that of Wollenweber & Reinking 1935 [6].

c? After the taxon - concept may be doubtful

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The collaborate reduce these differs. 1. They want by conducting as cal, genetical, an sible with relevan investigations, the three species,

# Material and me

Strains of F. samb orators of the Eur and between Octo 140 Fusarium same at the Institute Most of them w Europe. I typifie ly acquired taxon bucinum s. 1. ar groups (Table 2).

Of the 140 st study in the EFS ciation to the gro origin, their cultu ture), and their cc

Fourteen strai from group II, si into group IV, re typified properly. similarities with 1 of F. bactridioide. sambucinum isol: pseudopulicaris ` group VI. Both project because ( sisted of two isc pulicaris (Fr. ex four species were trol: F. culmorui (Cooke) Sacc. (= Toussoun) and F. chosen because o those of group I was placed by W F. sarcochroum reason was a cer charcteristics of agar.

The collaborators of the EFS-project intended to reduce these different views on Fusarium sambucinum's. 1. They wanted to offer an acceptable concept by conducting as many morphological, physiological, genetical, and biological investigations as possible with relevant strains. The results of one of these investigations, the morphological characterisation of the three species, is presented here.

#### Material and methods

Strains of F. sambucinum were requested from collaborators of the European F. sambucinum (EFS) project and between October 1987 and December 1988 about 140 Fusarium sambucinum s. 1. strains were sent to me at the Institute of Microbiology in Berlin-Dahlem. Most of them were isolated from potato tubers in Europe. I typified them according to my previously acquired taxonomic knowledge of Fusarium sambucinum s. 1. and arranged them in three different groups (Table 2).

Of the 140 strains, 41 were chosen for detailed study in the EFS-project bearing in mind their association to the group, their host plant, their country of origin, their cultural state (wild type, degenerated culture), and their colour on wort agar or PDA.

Fourteen strains were chosen from group I, eight from group II, six from group III. Five were placed into group IV, representing strains that could not be typified properly, but whose conidia showed certain similarities with those of group I-III. The exholotype of F. bactridioides represented group V, one strain of F. sambucinum isolated from the teleomorph Gibberella pseudopulicaris Wollenw. by E. Müller, represented group VI. Both cultures were incorporated into the project because of Booth's concept. Group VII consisted of two isolates of the teleomorph Gibberella pulicaris (Fr. ex Fr.) Sacc. One strain for each of the four species were meant to serve as some kind of control: F. culmorum (W. G. Smith) Sacc., F. cerealis (Cooke) Sacc. (=F. crookwellense Burgess, Nelson et Toussoun) and F. compactum (Wollenw.) Gordon were chosen because of the similarities of their conidia with those of group III, and F. lateritum Nees because it was placed by Wollenweber and Reinking along with F. sarcochroum into the section Lateritum. Another reason was a certain similarity between the cultural charcteristics of F. lateritium and group II on wort agar.

The 41 Fusarium cultures (Table 2) were grown on a synthetic medium with a low nutrient content (SNA) [9] in the laboratory at ca. 20–22 °C and natural day-night-rhythmn, in the dark (D) and under constant black light illumination (DL) at 20 °C for 10 to 28 days. The morphological characteristics were photographed. For the characterisation of the three species within F. sambucinum s. 1. (identical with group I–III) measurements were taken of four representative cultures of each group. Their means including standard deviations are given in the description as well as the extremes in parentheses. Since the strains are cultivated under standardised conditions [9] the variation of the size of the conidia is limited and measurements of more than 4 × 30 conidia will add no further information.

Growth habits, colour [10] and the cardinal temperatures (minimum, optimum, maximum) were taken from cultures grown on PDA in the laboratory or in the dark, respectively.

## Results

The differentiation of the *F. sambucinum* s. 1. strains into three groups proved to be correct. Of the originally 140 strains 53% belonged to group I, 20% to group II and 19% to group III. Therefore three species: *F. sambucinum* s. str., *F. torulosum comb. nov.*, and *F. venenatum* sp. nov. which correspond to the groups respectively are described and delimited.

F. sambucinum Fuckel s. str. - Symb. Mycol. 1869; 167

- = F. roseum Link (nomen ambiguum) Diss. I. Mag Ges naturf Freunde, Berl 1809; 7: 25–45.
- = F. sulphureum Schlecht. sensu Wollenw. Flora Berol 1824; 139.
- = F. trichoteciodes Wollenw. in Jamieson & Wollenweber, J Wash Acad Sci 1912; 2: 146–152.
- F. sambucinum Fuckel f. 2 Wollenw. Z Parasitenk 1931; 3: 357.
- = F. sambucinum Fuckel f. 6 Wollenw. Z Parasitenk 1931; 3: 358.
- = F. sambucinum Fuckel var. minus Wollenw. Z Parasitenk 1931; 3: 358.
- = F. roseum Link ex Gray emend. Snyder & Hansen pr. p. Am J Bot 1945; 32: 663

Teleomorph: Gibberella pulicaris (Fr. ex Fr.) Sacc. – Michelia 1877; 1: 43

Table 2. Test strains used in the European Fusarium sambucinum Project

	Collection number	Species	Colours of culture on wortager	Matrix	Country of origin	Collector or deposito
	Group I			<del>-</del> ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		
	64 995	F. sambucinum	yellowish	Brassica oleracea	Netherlands	Noordeloos
	64 678	F. sambucinum	yellowish	Triticum aestivum	Switzerland	Thrane
	62 433	F. sambucinum	yellowish	Beta vulgaris	Spain	Gerlach
	64 960	F. sambucinum	yellowish	soil	Denmark	Thrane
	64 747	F. sambucinum	rose	Soianum tuberosum	Fed. Rep. Germany	Langerfeld
	65 009	F. sambucinum	rose	Solanum tuberosum	Italy (?)	Logrieco
	62 434	F. sambucinum	rose	Solanum tuberosum	Iran	Gerlach
-0 V	64 226	F. sambucinum	rose	Solanum tuberosum	Great Britain	Nirenberg
87	64 998	F. sambucinum	rose	Solanum tuberosum	France	Tivoli
	64 996	F. sambucinum	brownish	Solanum tuberosum	France	Tivoli
- · ·	62 397	F. sambucinum	red	Solanum tuberosum	Fed. Rep. Germany	Gerlach
<b>8</b> 1	64 484	F. sambucinum	red	Solanum tuberosum	Finland	Seppänen
	64 262	F. sambucinum	red	Glycine max	Brazil	Nirenberg
	64 480	F. sambucinum	red	Solanum tuberosum	Finland	Seppänen
	Group II					**
	64 990	F. torulosum	red to brown	Buxus sempervirens	Netherlands	Noordeloos
	64 948	F. torulosum	brownish	unknown	Denmark	Thrane
	64 479	F. torulosum	brownish	Solanum tuberosum	Finland	Seppänen
	64 645	F. torulosum	whitish	Solanum tuberosum	Denmark	Thrane
	64 988	F. torulosum	red	Hordeum vulgare	Hungary	Széczi
/4	<b>62</b> 398	F. torulosum	red	Betula verrucosa	Fed. Rep. Germany	Gerlach
100	63 933 ·	F. torulosum	red	Triticum aestivum	Australia	Gerlach
,	64 465	F. torulosum	beige	Triticum aestivum	Fed. Rep. Germany	Nirenberg
	Group III		•		1	
950	64 935	F. venenatum	reddish	Solanum tuberosum	Poland	Latus
910	65 030	F. venenatum	reddish	Zea mays	Fed. Rep. Germany	Nirenberg
س نارور	64 757	F. venenatum	reddish	Humulus lupulus	Fed. Rep. Germany	Gerlach
99	64 537	F. venenatum	reddish	Triticum aestivum	Austria	Nirenberg
93	<b>/</b> 64 478	F. venenatum	brownish	Solanum tuberosum	Finland	Seppänen
12	65 031	F. venenatum	beige	Zea mays	German Dem. Rep.	Nirenberg
	Group IV, st	rains resembling F. sai	mbucinum s. 1. morph	ologically		
	64 371	F sp.	yellowish	Curcurbita maxima	New Zealand	Laundon
	64 280	F. cf. sambucinum	brownish	Ruschia comosa	Fed. Rep. Germany	Reinecke
	64 993	F. sambucinum	brownish	unknown	Netherlands	Hermanides-Nijhof
	63 575	F. sp.	yellowish	Medicago sativa	New Zealand	Gerlach
	64 351	F. semitectum var. semitectum	beige	Helianthus annuus	Jugoslavia	Nirenberg

Type: Herbarium Fuckel, 1894, No. 595, in herbarium Barbey-Bossier (G), Fungi rhenani 241 (Fig. 4). Cardinal temperatures: 2.0, 25.0, 32.5 °C.

Colonies: Growing in concentric rings, fast, reaching 5.0 cm in diam. in five days at 20 °C in the dark on PDA, margins lobed (Fig. 1), degenerated

strains growing somewhat faster, with margins not lobed at all (Fig. 1).

Colony reverse: Yellowish white, brownish orange, greyish orange, ruby with brown dots. Aerial mycelium: Generally abundant, floccose to felt-like, white, yellowish white, to greyish orange.

Table 2. Co



Fig. 1. Reverse growing in the la str. (BBA 64 995 (BBA 64 993), I 65 416).

Sclerotia: at orange to t Sporulation: on sclero

Table 2. Continued

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Collection Species number		Colours of culture on wortager	Matrix	Country of origin	Collector or depositor	
Group V 63 602 Group VI	F. bactridioides	red ,	Cronartium ribicola	USA (Arizona)	Wollenweber	
63 714 Group VII	F. sarcochroum	beige	Viscum album	Switzerland	Gerlach	
64 918	G. pulicaris	rose	palm tree	Indonesia	Samuels	
62 719	G. pulicaris	yellowish-rose	Pterocarya fraxinifolia	Iran	Gerlach	
Group VIII,	strains functioning	as controls				
64 218	F. culmorum	reddish	Hordeum vulgare	Finland	Nirenberg	
64 545	F. cerealis	reddish	Triticum durum	Fed. Rep. Germany	Nirenberg	
64 640	F. compactum	red	herbs	France	Thrane	
64 225	F. lateritum	rose	Malus domestica	Fed. Rep. Germany	Nirenberg	

Fig. 1. Reverse of F. sambucinums. 1. cultures on PDA after 9 days growing in the lab.: Clockwise from the upper left: F. sambucinums. str. (BBA 64 995), F. sambucinums. str., degenerated culture variant (BBA 64 993), F. torulosum (BBA 65 417) and F. venenatum (BBA 65 416).

; with margins not

brownish orange, own dots. Aerial , floccose to feltreyish orange. Sclerotia: abundant in fresh isolates, of brownish orange to brown colour, shape cauliflower-like.

Sporulation: starting in the aerial mycelium, later on sclerotial stroma as sporodochia, tinged

greyish orange, brownish orange, brown, and ruby, depending on colour of the isolate.

Odour: In some strains faintly mushroom-like.

Conidiophores: At first arising as single lateral phialides, later in sporodochia or pionnotes, consisting of delicate, long multicelled stalks, that branch vigorously, terminating in phialides.

Phialides: Monophialidic, cylindrical to doliiform, often with a collarette, mostly 14.0–18.0  $\times$  3.8–4.5  $\mu$ m.

Conidia: On SNA rather uniform in size, compact, falcate, with a constricted apical cell and a distinct pedicellate basal cell, mostly 3- to 5-septate, in DL the 3-septate conidia prevailed (Fig. 2), in D the 5-septate ones did (Fig. 3), they measured:

3-septate DL (28.4-)30.6-37.2(-40.0) × (4.0-)4.2-5.2(-6.0)  $\mu$ m
3-septate D (24.0-)24.1-26.9(-28.0) × (4.0-)4.1-4.7(-5.0)  $\mu$ m
5-septate DL (34.0-)36.8-44.4(-44.8) × (4.4-)4.5-5.3(-5.5)  $\mu$ m
5-septate D (26.8-)29.1-32.9(-35.2) × (4.2-)4.6-5.4(-5.6)  $\mu$ m

Chlamydospores: Not found within 14 days growing on SNA in the dark.

Occurence: Beta vulgaris, Brassica oleracea, Glycine max, Pterocarya fraxinifolia, Solanum tuberosum, Triticum aestivum, Vicia faba, soil.

Delimitation: F. sambucinum is a fast growing species with ruby, greyish orange or brownish orange sub-

strate mycelium, width of sporodochial conidia on SNA in DL 4.9  $\mu$ m, in D 5.0  $\mu$ m, no chlamydospores are found within 14 days on SNA in D, growing margin weakly lobed or not at all; its morphology is unique and can not be confused with any other *Fusarium* species (see Table 3).

# F. torulosum (Berk. et Curt.) Nirenberg

- ≡ Fusidium torulosum Berk. et Curt. North Am Fungi, No. 679.
- = Fusoma torulosum (Berk. et Curt.) Sacc. Syll Fung 1886; 4: 220.
- = F. sclerodermatis Oudem. Nederl Kruidk Arch II 1889; 5: 516.
- = F. polymorphum Matruchot Rech Dével Mucéd 1892: 84–91 Table 7, Fig. 6-14.
- = F. subpallidum Sherb. Mem Cornell Univ Agric Exp Stn 1915; 6: 175.
- = F. sambucinum Fuckel var. coeruleum Wollenw. Ann Mycol 1917; 15: 55.
- = F. elegantum Pratt J Agr Res 1918; 8: 84.
- = F. sambucinum Fuckel f. 1 Wollenw. Z Parasitenk 1931; 3: 356.
- = F. sambucinum f. 5 Wollenw. Z Parasitenk 1931; 3: 358.
- = F. roseum Link ex Gray emend. Snyder & Hansen pr. p. Am J Bot 1945; 32: 663.
- = F. pezizoideum (Berk. et Curt.) Sacc. Syll Fung 1886; 4: 711.
- = F. rostratum Speg. in Wollenweber & Reinking, Die Fusarien, Berlin: Verlag Paul Parey, 1935: 76.

Teleomorph: ?Gibberella pulicaris (Fr.) Sacc. var. minor Wollenw. – Z Parasitenk 1931; 3: 356.

Cardinal temperatures: 2.0, 27.5, 37.5 °C.

Colonies: Growing in narrow concentric rings, slowly, reaching 2.0-2.5 cm in diam. in 5 days on PDA in the dark at 20 °C, margins lobed (Fig. 1).

Colony reverse: Ruby, with growing margin white, degenerated cultures may be yellowish white or brown.

Aerial mycelium: Short, dense, lannose to felt-like, orange white, shining greyish rose above the ruby mycelium of the substrate; growing margin 0.5-1.0 cm, pure white.

Sclerotia: Present bluish black, cauliflower-like in shape.

Sporulation: Starting comparatively late, usually in orange sporodochia.

Odour: Not perceptible.

Conidiophores: At first arising as single lateral phialides on the hyphae, later in sporodochia branching densely.

Phialides: Monophialidic, cylindrical to slightly doliiform, measuring  $14.0-18.0 \times 3.2-4.2 \mu m$ .

Conidia: On SNA from sporodochia, in DL slender, falcate, with a pointed apical cell and a distinct pedicillate basal cell (Fig. 5), in D relatively compact with a rounded apical cell and a truncate basal cell, mostly 5-septate (Fig. 6), measuring:

5-septate DL (32.0-)35.8-40.0(-42.0) × 4.0-4.4  $\mu$ m 5-septate D (28.8-)31.7-35.9(-37.0) × (4.4-)4.6-5.4(-5.6)  $\mu$ m.

Those in D resemble the conidia of the type specimen, F. bactridioides (Fig. 9). Often 0- to 1-septate conidia occur in DL and D (Fig. 7).

Chlamydospores: Formed in the dark on SNA usually in long chains or/and clusters (Fig. 8).

Occurence: Beta vulgaris, Betula verrucosa, Buxus sempervirens, Hordeum vulgare, Pinus sylvestris, Solanum tuberosum, Triticum aestivum, soil.

Delimitation: F. torulosum is a slow growing species with ruby substrate mycelium and a white, lobed growing margin, width of sporodochial conidia on SNA in DL 4.2  $\mu$ m, in D 4.8  $\mu$ m, often 0-and 1-septate microconidia are formed, chlamydospores are produced within 14 days on SNA in D, growth habit on wort agar similar to cultures of F. lateritium or F. flocciferum Corda, not considering colour (see Table 3).

## F. venenatum sp. nov.

- = F. sambucinum Fuckel var. coeruleum Wollenw. sensu Booth The genus Fusarium 1971: 171-172.
- = F. sambucinum Fuckel var. coeruleum Wollenw. sensu Gerlach & Nirenberg – Mitt Biol Bundesanst Land- Forstw 1982; 209: 213–216.
- =? F. sulphureum Schlecht. Flora Berol 1824; 139.
- =? F. culmorum W.G. Smith var. cerealis (Cooke) Wollenw. pr. p. Z Parasitenk 1931; 3: 362.
- =? F. equiseti (Corda) Sacc. var. crassum Wollenw. - Z Parasitenk 1931; 3: 333.
- = F. roseum Link. emend. Snyder & Hansen pr. p. -Am J Bot 1945; 32: 663.

Coloniae in agaro PD 20 °C in 5 diebus circiter 44 mm diametro, candidae usquae pallide-roseae plerumque rubrae centro flavae, lannosae; reverso rubrae, rario



Fig. 2. F. sambucinus conidia on SNA in dari



Fig. 5. F. torulosum; on SNA in darkness at

ig as single lateral i sporodochia branch-

frical to slightly doli-3.2-4.2 μm. Schia, in DL slender, i cell and a distinct in D relatively coml and a truncate basal measuring:

40.0(-42.0) ×

i5.9(-37.0) × 4(-5.6) μm.

idia of the type spec-9). Often 0- to 1d D (Fig. 7). dark on SNA usually (Fig. 8). da verrucosa, Buxus are, Pinus sylvestris,

aestivum, soil. slow growing species 1 and a white, lobed sporodochial conidia D 4.8 μm, often 0e formed, chlamydo-4 days on SNA in D, similar to cultures

n Corda, not consider-

coeruleum Wollenw.

coeruleum Wollenw.
Mitt Biol Bundesanst
3-216.

Flora Berol 1824; 139. var. cerealis (Cooke) ik 1931; 3: 362.

ar. crassum Wollenw.

der & Hansen pr. p.

diebus circiter 44 mm ide-roseae plerumque reverso rubrae, rario

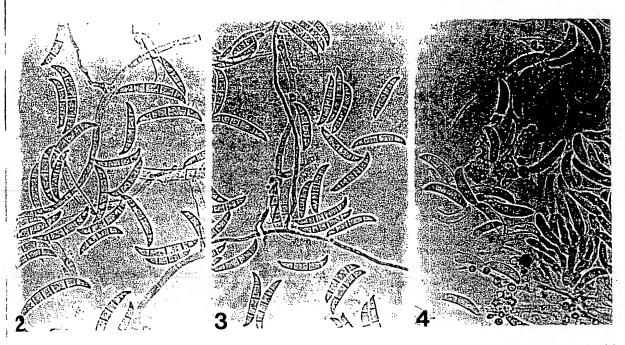


Fig. 2. F. sambucinum; sporodochial conidia on SNA under continuous black light at 20 °C; × 500. Fig. 3. F. sambucinum; sporodochial conidia on SNA in darkness at 20 °C; × 500. Fig. 4. F. sambucinum, type (G); sporodochial conidia from Sambucus nigra; × 500.

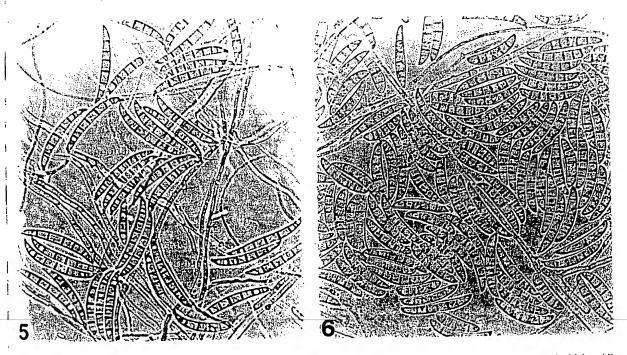


Fig. 5. F. torulosum; sporodochial conidia on SNA under continuous black light at 20 °C;  $\times$  500. Fig. 6. F. torulosum; sporodochial conidia on SNA in darkness at 20 °C;  $\times$  500.

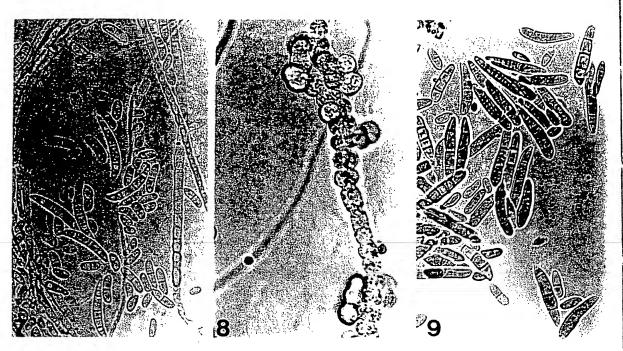


Fig. 7. F. torulosum; sporodochial conidia and microconidia under continuous black light at 20 °C; × 500. Fig. 8. F. torulosum; chains of chlamydospores on SNA in darkness at 20 °C, × 500. Fig. 9. F. bactridioides, type (NY); sporodochial conidia from Cronartium ribicola; × 500.

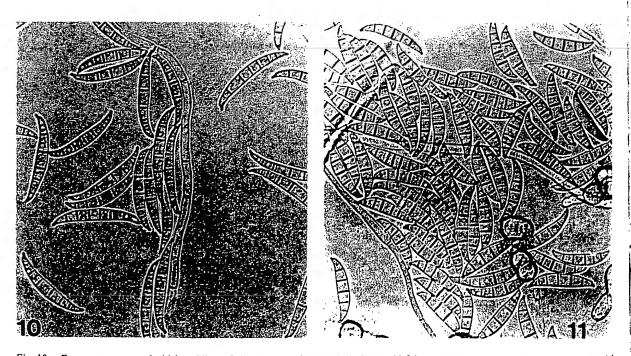


Fig. 10. F. venenatum; sporodochial conidia on SNA under continuous black light at 20 °C; × 500. Fig. 11. F. venenatum; sporodochial conidia and chlamydospores on SNA in darkness at 20 °C; × 500.

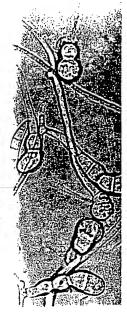


Fig. 12. F. venenatum; c potato tuber;  $\times$  500.

Table 3. Morpl

Species

F. sambucinus. s. str.
F. torulosum

F. veneņatun

cremeae. Tempera Conidia in mycelic lata in sporodochii na, falciformia, ap 5-septata, in agerc (-42.4) × (4.8-)5... 36.4-40.0(-42.0) > phora simplicia v

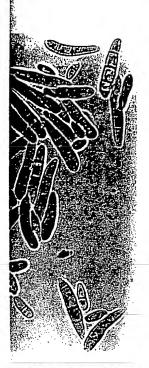


Fig. 8. F. torulosum; chains of a from Cronartium ribicola; ×

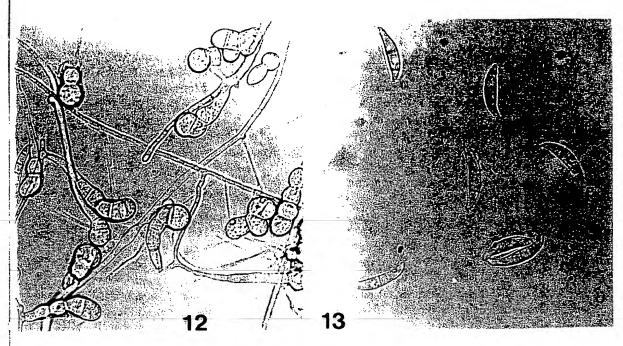


Fig. 12. F. venenatum; chlamydospores on SNA in darkness at 20 °C; × 500. Fig. 13. F. sulphureum, type (HAL); sporodochial conidia from potato tuber; × 500.



'. F. venenatum; sporodochial

Table 3. Morphological and physiological differences within Fusarium sambucinum s. 1.

Species	Growth at 20 °C	Cardinal temperatures	Micro- conidia	Size of 5-septate conidia on SNA	Size of 5-septate conidia on SNA	Chlamydospores
	on PDA (mm/day)	(°C)		x	in DL (μm) x̄	in D (μm)
	(шпицау)					
F. sambucinum s. str.	5.0 <sup></sup>	2.0, 25.0, 32.5	(-)	40.6 × 4.9	31.0 × 5.0	-
F. torulosum	2.2	2.0, 27.5, 37.5	+	37.9 × 4.2	33.8 × 4.8	Intercalary, in long chains
F. venenatum	4.4	2.0, 25.0, 35.0	-	38.6 × 5.5	38.4 × 6.3	Laterally, in short, hooked chains

cremeae. Temperationes cardinales 2.0, 25, 35 °C. Conidia in mycelio aerio nulla, conidia etiam accumulata in sporodochiis brunneis vel bruneo-rubris, hyalina, falciformia, apice acerosa, pedicellata, plerumque 5-septata, in agero SN et luce (DL) (35.4-)37.0-40.2 (-42.4)  $\times$  (4.8-)5.1-5.9(-6.2)  $\mu$ m, obscure (D) (34.2-)36.4-40.0(-42.0)  $\times$  (5.2-)5.7-6.9(-7.4)  $\mu$ m. Conidiophora simplicia vel ramosa cum phialidibus simpli-

cibus, doliiformibus. Sclerotia et odor absunt. Chlamy-dosporae singulae in hyphis, saepe lateraliter catenis brevibus curvatis, griseae.

Habitat: in culmis Tritici aestivi.

Aera geographica: Austria.

Holotypus: in culto agaro desiccatus Fusarii venenati Nirenberg(CBS 458.93).

Exholotypus: BBA 64 537, CBS 458.93.

Cardinal temperatures: 2.0, 25.0, 35.0 °C.

Colonies: Growing in concentric rings, reaching 4.3-4.6 cm in diam. in 5 days on PDA in the dark at 20 °C, margins lobed (Fig. 1).

Colony reverse: Ruby to reddish brown, seldom greyish orange, degenerated cultures often brown.

Aerial mycelium: Long, dense, cottony white, orange grey, or ruby, in the middle of the colony sometimes brownish orange.

Sclerotia: Not found.

Sporulation: In brown to reddish brown sporodochia.

Odour: Not perceptible.

Conidiophores: At first arising as single lateral phialides on hyphae, soon branching generously, terminating in phialides.

Phialides: Monophialidic, mostly dolliform, measuring  $12.0-20.0 \times 4.8-5.0 \mu m$ .

Conidia: On SNA in DL relatively slender, falcate with a pointed apical cell and a pedicellate basal cell (Fig. 10), in D wider, resembling conidia of F. cerealis, F. compactum or F. sulphureum (Fig. 13), mostly 5-septate (Fig. 11), measuring:

5-septate DL (35.4-)37.0-40.2(-42.4) × (4.8-)5.1-5.9(-6.2)  $\mu$ m. 5-septate D (34.2-)36.4-40.4(-42.0) × (5.2-)5.7-6.9(-7.4)  $\mu$ m.

Chlamydospores: On SNA in D pale to light grey coloured, single or in lateral hooked, short chains (Fig. 12).

Occurence: Humulus lupulus, Solanum tuberosum, Spinacia oleracea, Triticum aestivum, Zea mays, soil.

Delimitation: F. venenatum is a fast growing species with intenstive ruby substrate mycelium, lobed growing margin, width of sporodochial conidia on SNA in DL 5.5  $\mu$ m, in D 6.3  $\mu$ m, resembling those of F. cerealis and F. compactum in D or conidia of the type specimen of F. sulphureum, chlamydospores pale grey coloured, mostly in lateral somewhat hooked short chains (see Table 3).

Distribution: Europe.

Holotypus: Dried culture of F. venenatum Nirenberg (CBS 458.93).

Exholotypus: BBA 64 537, CBS 458.93.

Of group IV, only two isolates could be identified in the course of the project: BBA 64 993 as F. sambucinum

and BBA 64 351 as *F. semitectum* Berk. et Rav. var. semitectum. The identity of the others in that group remain unsolved. *F. bactridioides* (group V) looks very similar to *F. torulosum* (Fig. 6, 9), but since the only existing culture is old and degenerated, a questionmark has to remain. The one strain (BBA 63 714) of *F. sarcochroum* (group VI) does not look like one of the three described species and has to be accepted as a species of its own. The two isolates originating from *G. pulicaris* (group VII) are designated as *F. sambucinum* s. str. The morphology of the four control strains did not match with any other strain used in this project.

#### Discussion

Since the three taxa exhibited unique morphological features, and no strong affinities between them could be found otherwise, all of them were given species rank: *F. sambucinum* s. str., *F. torulosum* and *F. venenatum*. Degenerated cultures of *F. sambucinum* s. str. and of *F. torulosum*, however, become difficult to identify, since both can produce slender sporodochial conidia and 0-to 1-septate microconidia (= sign of degeneration in *F. sambucinum* s. str.). BBA 64 993 represents such a culture (Fig. 1).

It could be demonstrated that *F. sulphureum* is not a synonym for *F. sambucinum* s. str. as Wollenweber & Reinking [6] thought. *F. sulphureum* might be synonymous with either *F. cerealis* or *F. venenatum*. Only rDNA sequencing or RAPD PCR of the type material will resolve this question. Since *F. sulphureum* was used for such a long time in a different sense, it will probably become a nomen dubium such as *F. roseum*.

The question as to whether F. bactridioides and/or F. sarcochroum are synonyms of F. sambucinum could not be solved by their morphological characteristics. The answers might be found by investigations in which methods of molecular biology are employed.

O'Donnell [11] sequenced the ITS region of F. sambucinum rDNA and found three types (A, B, C) with quite high divergence between them. Type B coffresponds with the F. sambucinum s. str. strains! If they belong to one mating population, as he stated, it has to be assumed, that types A and C are different, non-European populations within the species F. sambucinum s. str.

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Acknowledgement

The author thanks N skillful technical as

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2. Domsch KH, Gar fungi, Vol. 1. Lon

 Gerlach W, Nirer atlas. Mitt Biol Bi

 Marasas WFO, N ium species: I Park/London: Per

5. Snyder WC, Han with reference to . 32: 657-666.

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m Berk. et Rav. var. others in that group (group V) looks very ), but since the only rated, a questionmark (BBA 63 714) of F. I look like one of the to be accepted as a es originating from G. ted as F. sambucinum for control strains did sed in this project.

inique morphological between them could be re given species rank: um and F. venenatum. ucinum s. str. and of F. ficult to identify, since lochial conidia and 0-gn of degeneration in 993 represents such a

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F. bactridioides and/or f F. sambucinum could logical characteristics. investigations in which are employed.

the ITS region of F. three types (A, B, C) veen them. Type B cornum s. str. strains. If pulation, as he stated. as A and C are differs within the species F.

# Acknowledgements

The author thanks Mrs Heidrun Anders for her reliable, skillful technical assistance.

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